Structures of a New Limonoid and a New Triterpenoid Derivative from Pericarps of Trichilia connaroides

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STRUCTURES OF A NEW LIMONOID AND A NEW TRITERPENOID DERIVATIVE FROM PERICARPS OF *TRICHILIA CONNAROIDES*

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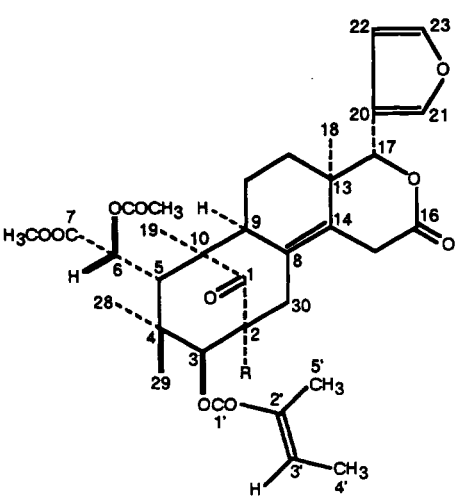
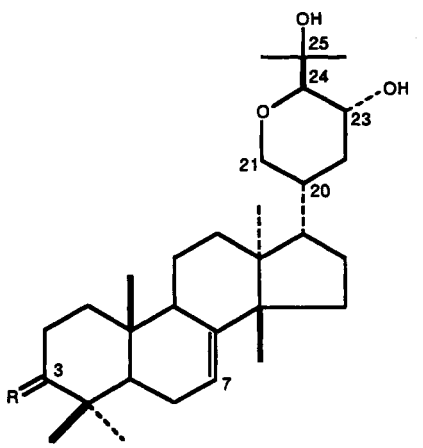
ABSTRACT.—A new mexicanolide-type limonoid, 2-hydroxy-3-*O*-tigloyl-6-*O*-acetylswietenolide [**1**], and a new tirucallane-type triterpenoid derivative, lipo-3-episapelin A [**2**], were isolated from pericarps of *Trichilia connaroides* and their structures established on the basis of chemical and spectral evidence. In addition, five known tirucallane-type triterpenoids were also isolated.

Roots of *Trichilia connaroides* (Wight et Arn.) Bentv. (1) [*Heynea trijuga* Roxb. var. *multijuga* Roxb. (2)] (Meliaceae) are used as a Chinese crude drug to treat arthritis, pharyngitis, tonsillitis, and other ailments (3). Previously, two cycloartane-type triterpenoids (4), and three limonoids (5,6), have been characterized from the leaves and flowers of this plant. As part of our studies on the constituents of plants of the Meliaceae, we have now examined the pericarps of *T. connaroides*. As a result, we have obtained a new mexicanolide-type limonoid and a new tirucallane-type triterpenoid derivative, namely, 2-hydroxy-3-*O*-tigloyl-6-*O*-acetylswietenolide [**1**] and lipo-3-episapelin A [**2**], respectively. In the present paper, the structural determination of these new compounds is described.



1 R=OH
3 R=H



2 R=αH, βOCO$(CH_2)_nCH_3$ (n=10, 12, 14, 16)
4 R=αH, βOH

After cc and hplc separations of the $CHCl_3$-MeOH (50:1)-soluble part of the MeOH extract, compounds **1** and **2** were isolated, together with five known triterpenoids, melianone (7), melianol (7), lipomelianol (7), melianodiol (8), and dihydroniloticin (9).

2-Hydroxy-3-*O*-tigloyl-6-*O*-acetylswietenolide [**1**], colorless oil, had a molecular formula of $C_{34}H_{42}O_{11}$ (M^+ 626.273, calcd 626.273) by hreims data and showed strong absorptions at 1730 cm^{-1} (δ-lactone), 1720 cm^{-1} (ester), 1700 cm^{-1} (ketone), 1600, 1510, and 875 cm^{-1} (furan) in the ir spectrum. The ^{1}H-nmr spectrum of **1** (Table 1), analyzed with the aid of 2D nmr studies (COSY and NOESY) indicated the presence of four tertiary methyls (δ 0.89, 1.02, 1.04, and 1.28), a methyl ester (δ 3.77), an acetoxyl

TABLE 1. ^{1}H- and ^{13}C-Nmr Spectra of **1** and the ^{13}C-Nmr Spectrum of **3** (11) (in $CDCl_3$).[a]

Position	Compound			Position	Compound		
	1		3		1		3
	δ_H	δ_C	δ_C		δ_H	δ_C	δ_C
1		216.68 s	217.32 s	18	1.02 s	18.66 q	17.50 q
2		78.10 s	48.16 d	19	1.28 s	17.44 q	16.80 q
3	4.84 s	87.16 d	80.10 d	20		120.66 s	120.75 s
4		39.83 s	39.30 s	21	7.54 dd, 1.5,1	143.09 d	141.48 d
5	3.51 s	44.17 d	44.21 d				
				22	6.46 dd, 2,1	109.90 d	109.94 d
6	5.50 s	72.84 d	73.14 d	23	7.44 dd, 2,1.5	141.50 d	143.03 d
7		171.28 s	171.41 s	28	1.04 s	22.30 q	23.14 q
8		125.59 s	127.67 s	29	0.89 s	23.25 q	23.66 q
9	2.06 m	52.79 d	53.14 d	30	1.75 d,14	44.64 t	33.92 t
10		52.63 d	53.56 s		3.04 d,14		
11	2.0-2.2 m	18.66 t	18.71 t	7-OCH_3 ..	3.77 s	53.20 q	53.14 q
12	1.16–1.24 m	29.36 t	29.46 t	$OCOCH_3$.	2.19 s	20.95 q	20.99 q
13		38.31 s	38.17 s	$OCOCH_3$..		169.73 s	169.80 s
14		133.76 s	132.49 s	1′		167.08 s	167.25 s
15	3.25 dt, 21,3	33.18 t	33.07 t	2′		129.16 s	128.97 s
	3.61 dd, 21,1.5			3′	6.92 qq, 7,1	139.13 d	139.39 d
16		169.13 s	169.38 s	4′	1.82 qd, 7,1	14.54 q	14.61 q
17	5.47 s	80.81 d	81.01 d	5′	1.90 d 1	12.43 q	12.34 q

[a]Chemical shifts are expressed as δ values and are followed by multiplicities and coupling constants (Hz).

group (δ 2.19), a β-substituted furan ring, and a tigloyl group. In addition, the ^{13}C-nmr spectrum of **1** (Table 1) exhibited signals due to a ketone (δ 216.68), four carbonyls (δ 167.08, 169.13, 169.73, and 171.28), a tetrasubstituted double bond (δ 125.59 and 133.76), a β-substituted furan ring, and four tertiary methyls. The above spectral data suggested that **1** is a member of the mexicanolide group of limonoids (10), and the ^{1}H-nmr data of **1** were very similar to those of 3-*O*-tigloyl-6-*O*-acetyl-swietenolide [**3**] (11) isolated from seeds of *Swietenia mahagoni* (Meliaceae). However in **1**, the signals due to H-3 (δ 4.84) and H_2-30 (δ 1.75 and 3.04) appeared as a sharp singlet and a pair of doublets (J=14.0 Hz), respectively, suggesting that C-2 in **1** is fully substituted, whereas in **3**, these protons appeared as a doublet and a pair of double doublets. The molecular formula of **1**, which contains one more oxygen atom than **3** indicated that C-2 in **1** is substituted by a hydroxy group. This was also substantiated by a hydroxy group absorption (3500 cm^{-1}) in the ir spectrum. The ^{13}C-nmr studies (Table 1) analyzed with the aid of HETCOR and COLOC experiments confirmed the structure. As shown in this table, the chemical shifts of **1** closely resembled those of **3** (11), but revealed a large downfield shift of the C-2 signal (δ 78.10) and other downfield shifts of the C-3 (δ 87.16) and C-30 signals (δ 44.64) as compared to **3** (δ 48.16, 80.10, and 33.92, respectively). On the basis of these spectral data, the structure of **1** was defined. The absolute stereochemistry of **1** was shown to be the same as that of swietenine (11,12) based on the negative Cotton effect at around 288 nm in the cd spectrum.

Lipo-3-episapelin A [**2**], colorless fine plates, mp 113-116°, showed typical ester absorption (1720 cm^{-1}) in the ir spectrum and gave four molecular ion peaks at *m/z* 740, 712, 684, and 656 in the fdms. The ^{1}H-nmr spectrum of **2** showed signals due to a vinyl proton (δ 5.27, dd, J=7 and 3 Hz), five protons geminal to an oxygen atom, and seven tertiary meth-

TABLE 2. ^{13}C-Nmr Spectrum of **2** (in $CDCl_3$).[a]

Position	δ_C	Position	δ_C	Position	δ_C
1	34.88 t	11	18.03 t	21	70.11 t
2	24.24 t	12	33.01 t	22	36.51 t
3	80.78 d	13	43.35 s	23	64.63 d
4	37.92 s	14	51.29 s	24	86.52 d
5	50.84 d	15	33.90 t	25	74.23 s
6	23.79 t	16	27.44 t	26	24.01 q
7	117.93 d	17	44.81 d	27	28.54 q
8	145.61 s	18	22.27 q	28	27.61 q
9	48.82 d	19	13.16 q	29	15.94 q
10	34.85 s	20	37.51 d	30	27.31 q
				$CH_3(CH_2)nCO$	173.71 s
				$CH_3(CH_2)nCO$	14.10 q

[a]Chemical shifts are expressed as δ values and are followed by multiplicities.

yls. The ^{13}C-nmr spectrum of **2** (Table 2) gave signals for an ester carbonyl (δ 173.71), a trisubstituted double bond (δ 117.93 and 145.61), five carbons bearing an oxygen atom (δ 64.63, 70.11, 74.23, 80.78, and 86.52), and seven tertiary methyls. Alkaline hydrolysis of **2** gave the known Δ^7-tirucallane-type triterpenoid, 3-episapelin A [**4**] (13,14), mp 211-213°, and a mixture of fatty acids. The composition of the acid units was clarified as follows. The eims and hreims of the mixture indicated the presence of stearic, palmitic, myristic, and lauric acids. In addition, the corresponding methyl ester mixture was analyzed by gc-ms, and its components were identified as methyl stearate, palmitate, myristate, and laurate in a ratio of 8:14:67:11. These results indicated that **2** is an ester mixture consisting of **4** and four different fatty acids. Further, the H-3α (δ 4.52, dd, J=11 and 4 Hz) and C-3 (δ 80.78) resonances of **2** exhibited downfield shifts (by ca. 1.3 ppm and 1.5 ppm, respectively) compared with those (δ 3.24 and 79.27) of **4** showing that the fatty acids are connected to the OH-3β of **4** through an ester bond. Based on this evidence, lipo-3-episapelin A was defined as a mixture (8:14:67:11) of the 3-*O*-stearate, -palmitate, -myristate, and -laurate of 3-episapelin A [**4**], as represented by **2**.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All mps were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. Ir spectra were run with a Jasco A-302 instrument. ^{1}H- and ^{13}C-nmr spectra were recorded with a JEOL-GSX 400 spectrometer (400 and 100.5 MHz, respectively) in $CDCl_3$. Optical rotations and the cd spectrum were measured for solutions in $CHCl_3$ on a Jasco DIP-140 digital polarimeter and a Jasco J-5000 spectropolarimeter, respectively.

The eims, hreims, and fdms (carbon emitter; accelerating voltage, 3kV; emitter current 15-29 mA) were taken with a JEOL JMS DX-300 mass spectrometer. Gc-ms were recorded with a combination of a JEOL MSGC-05 gas chromatograph (column, 10% SILAR-10C on Uniport HP; column temperature 140–230° at 8°/min) and a JEOL JMS DX-300 mass spectrometer. Prep. hplc was carried out on a Waters instrument with a M 6000A pump, a U6K septum-less injector, a series R-401 differential refractometer and a Si gel column (Waters μ-Porasil; 7.8 mm × 30 cm), with $CHCl_3$/MeOH or hexane/EtOAc as eluents.

PLANT MATERIAL.—Fruits of *T. connaroides* were collected at the Botanical Gardens of the University of Tokyo (Faculty of Science) in October 1987, and voucher specimens have been deposited in the herbarium of the Faculty of Pharmaceutical Sciences, Setsunan University.

EXTRACTION AND ISOLATION.—The dried fruits were separated into pericarps and seeds. Crushed pericarps (25 g) were then extracted with MeOH (600 ml×3) and the solvent was evaporated. The $CHCl_3$-MeOH (50:1)-soluble part (4.6 g) of the MeOH extract (7.0 g) was chromatographed on Si gel and the fractions were further purified by repeated hplc to afford **1** (11 mg), **2** (43

mg), and five known tirucallane-type triterpenoids. These triterpenoids were determined to be melianone (7), melianol (7), lipomelianol (7), melianodiol (8), and dihydroniloticin (9) by means of spectroscopic analysis.

2-Hydroxy-3-O-tigloyl-6-O-acetylswietenolide [**1**].—Colorless oil; $[\alpha]^{20}D$ −69.1° (*c*=0.20); ir ν max ($CHCl_3$) 3500 (OH), 2925, 1730 (δ-lactone), 1720 (ester), 1700 (ketone), 1600, 1510, 1280, 1240, 1130, 1060, 875 cm^{-1}; eims and hreims *m/z* 626.273 (M^+, calcd for $C_{34}H_{42}O_{11}$ 626.273, 5), 530.251 (calcd for $C_{29}H_{38}O_9$ 530.252, 37), 502.256 (calcd for $C_{28}H_{38}O_8$ 502.257, 72), 484 (23), 430 (61), 402 (25), 370 (40), 305 (73), 299 (33), 287 (79), 271 (31), 195 (92), 83 (100); ^1H and ^{13}C nmr, see Table 1; cd (*c*=4.7×10^{-3}) [θ] (nm) -2.83×10^3 (288) (negative maximum).

Lipo-3-episapelin A [**2**].—Colorless fine plates; mp 113–116° (hexane/Et_2O); $[\alpha]^{20}D$ +20.5° (*c*=0.19); ir ν max ($CHCl_3$) 3450 (OH), 2950, 1720 (ester), 1465, 1380, 1175, 1110, 1075, 1000 cm^{-1}; eims *m/z* 740 (M^+, 19), 712 (M^+, 38), 684 (M^+, 44), 656 (M^+, 45); ^1H nmr δ 5.27 (dd, *J*=7 and 3 Hz, H-7), 4.52 (dd, *J*=11 and 4 Hz, H-3α), 3.95 (d, *J*=11 Hz) and 3.39 (dd, *J*=11 and 4 Hz) (H_2-21), 3.92 (m, H-23β), 2.90 (d, *J*=9 Hz, H-24α), 1.31, 1.28, 1.00, 0.94, 0.85, 0.78, 0.77 (each 3H, s, 7 × tertiary methyls), 0.88 [3H, t, *J*=7 Hz, $Me(CH_2)_nCOO$- (n=10, 12, 14, and 16)]; ^{13}C nmr, see Table 2.

Alkaline hydrolysis of **2**.—A solution of compound **2** (32 mg) in 5% KOH/MeOH (4 ml) was stirred at room temperature for 24 h. The reaction mixture was poured into ice H_2O neutralized with 5% aqueous HCl, and extracted with Et_2O. After evaporation of the solvent, the residual product mixture was separated by hplc to give 3-episapelin A [**4**] (13,14) (15 mg), mp 211–213° [lit. (14) mp 204–205°]; $[\alpha]^{20}D$ +5.3° (*c*=0.22) [lit. (14) −5.2°]; ir ν max ($CHCl_3$) 3475 (OH), 2960, 2880, 1465, 1080 cm^{-1}; hreims *m/z* 474.371 (M^+, calcd for $C_{30}H_{50}O_4$ 474.371, 71); ^1H nmr δ 5.27 (dd, *J*=7 and 3 Hz, H-7), 3.95 (d, *J*=11 Hz) and 3.39 (dd, *J*=11 and 4 Hz) (H_2-21), 3.91 (m, H-23β), 3.24 (dd, *J*=11 and 4 Hz, H-3α), 2.90 (d, *J*=9 Hz, H-24α), 1.31, 1.28, 1.00, 0.97, 0.86, 0.79, 0.75 (each 3H, s, 7 × tertiary methyls) and a mixture of four higher fatty acids (8.7 mg); eims and hreims *m/z* 284.271 (M^+ for stearic acid, 1) (calcd for $C_{17}H_{35}COOH$, 284.271), 256.240 (M^+ for palmitic acid, 14) (calcd for $C_{15}H_{31}COOH$, 256.240), 228.209 (M^+ for myristic acid, 3) (calcd for $C_{13}H_{27}COOH$, 228.209), 200.179 (M^+ for lauric acid, 5) calcd for $C_{11}H_{23}COOH$, 200.178). This acid mixture was converted by CH_2N_2 treatment into a mixture of the corresponding methyl esters, the composition of which was determined by gc-ms to be methyl stearate, palmitate, myristate, and laurate in a ratio of 8:14:67:11.

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